Electronic Supplementary Material (ESI) for Soft Matter.

Electronic Supporting Information: Fluorescence-activated cell sorting (FACS) for purifying colloidal clusters

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1 Extended Methods

1.1 sCAPA

At first, negatives for the sCAPA traps were prepared on Si wafers using a Nanoscribe Photonic Professional GT2. This was done by spin coating (30 s, 2000 rpm) a 1 µm film of SU-8 6001-TF on a plasma-cleaned 4-inch Si wafer followed by a pre-baking step on a 100 °C hot plate for 3 min. Using the Nanoscribe, the pattern was then written on the coated wafer with a 20x objective (laser power 75 %, writing speed 10000 µm $^{-1}$, two passes). Then, the wafer was baked on a hot plate at 110 °C for 2 min before it was developed for 3 minutes in PGMEA and cleaned with PGMEA and IPA. Hard-baking was done for 1.5 h at 175 °C. Subsequently, the developed wafer was placed in a vacuum chamber containing approximately 50 µL of trichloro(1H,1H,2H,2H-perfluorooctyl)silane (Sigma-Aldrich, > 98 %). The wafer was then rinsed with ethanol and dried with dry N2 before use. To prepare the traps, a PDMS-based elastomer (Dow Chemicals' SYLGARD 184 silicone elastomer) was poured over the silicon wafer and cured overnight at 80 °C in an oven. Red-fluorescent polystyrene (PS) beads (2.48 µm, Microparticles GmbH) and green-fluorescent polystyrene (PS) beads (2.8 µm, Microparticles GmbH) were used. The suspension for sCAPA was freshly prepared before each deposition. The surfactant concentration of the sCAPA suspension was optimized to give the right contact angle for single-particle deposition. The final suspension contained 2 mM sodium dodecyl sulfate (Sigma-Aldrich), 0.02 % w/w Triton X-45 (Sigma-Aldrich) for single-particle depositions and 6 mM sodium dodecyl sulfate, and 0.06 % w/w Triton X-45 for sideways depositions. Per deposition, 45 µl was used for single particle depositions and 90 µl for sideways depositions, and the droplet was moved over the substrate at 5 µm/s for single particle depositions and 2 µm/s for sideways depositions, respectively.

1.2 Sintering and Harvesting

After deposition, the filled traps were sintered at 110 °C for 15 min in an oven. The harvesting was done by the freezing droplet methods previously described by Ni et al. [1]. The filled traps were placed on a copper block, which was cooled down to -50 °C. Then, a 10 µl MQ droplet was placed on the particle area, and a pipette tip was placed inside the droplet. When the droplet froze, it was transferred to an Eppendorf tube containing 20 µl of 0.1 % w/w in MQ. This procedure was repeated 3 - 4 times until most of the patterned area was harvested.

1.3 Quantification

The quantification of the cluster sub-populations after harvesting was done from microscopy images (Nikon, Eclipse Ti-2). A droplet of the harvested suspension was placed on a glass slide with a spacer and sealed with a glass slide. An image array was taken and stitched together, before using an image analysis script programmed in Matlab. The script uses a single channel image for each particle type of interest (Red/Green), and one RGB combined image for illustration purposes. Briefly, the script works as follows: the in-built imfindcircle function uses a Circular Hough Transform-based algorithm to locate circles, which allows for determining the location of the particles. At first, the monomers and dimers were sorted by conditions on neighboring particles within a given radius (a particle with no neighbors is a monomer, and two particles next to each other with each having one neighbor are defined as dimers). Linear trimers were searched by the following procedure: starting with particles with one neighbor, it was checked if this neighbor itself had two neighbors and the third particle had only one neighbor and was aligned with the two particles already identified (¡ 60 °). This procedure was then adapted for tetramers, pentamers, and so on up to a particle length of ten. In the last step, all the clusters (non-linear particle groups)

were counted by adding all the neighbors of a particle to an array, adding the neighbors of the neighbors, and so on, and in the end, filtering the array to have only one entry per particle and determine the size of the corresponding cluster. Microscopy images were also used to quantify the sorted samples, but the particle suspensions after sorting were too diluted to be counted efficiently by the script. Therefore, they were manually counted manually, with a minimum of 100 colloidal clusters per set.

1.4 Additional details of FACS experiments

The colloidal clusters were sorted by FACS using a BD-FAC AriaIII with a 70 µm nozzle, which allows for sorting for four distinct populations. When sorting only for length and color, the red and green fluorescence lines were used. The trimers sorted for shape were gated first using the red fluorescence line as the first gate and the forward-scattering width as the secondary gate. For the sorting experiments, the initial suspension was diluted with 200 µof 0.1 % w/w Pluronic F-127 in MQ. The clusters were sorted into Eppendorf tubes containing 100 µl of aqueous 0.1 % w/w Pluronic F-127 solution.

2 Effect of sintering temperature

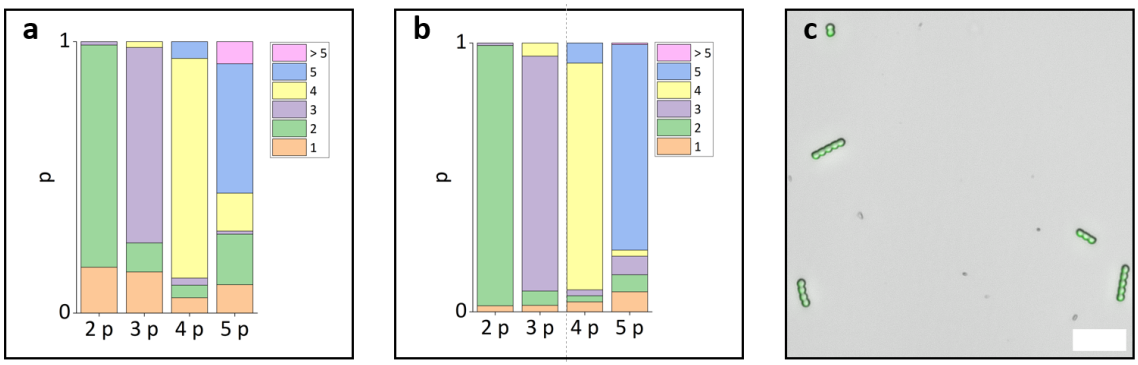

Figure S1: **Comparison of linear clusters up to five particles sintered at various temperatures** a) Image analysis data of FACS-sorted samples sintered at 100 °C. b) Image analysis data of FACS-sorted samples sintered at 110 °C. c) Combined bright-field and fluorescence microscopy image of a unsorted sample sintered at 120 °C. Scale bar is 20 µm.

The sintering step after sCAPA is crucial to prevent cluster breaking during handling and, thus, to achieve the best sorting results. The FACS procedure requires a strong bond between the particles, but the shape of the individual particles should remain intact. With a sintering temperature of 120 °C for 15 min in the oven, the particles clearly deformed, see the microscope image in Fig. S1 a. When sintered at 100 °C however, the population purity after the FACS was not as high as when sintered at 110 °C. For example, at 100 °, the sintered and sorted trimer sub-population had a purity of 72.0 %, while the same population sintered at 110 °C had a purity of 87.4 % (see Fig. S1 a, b). This leads to the hypothesis that stresses during the FACS procedure arise, which can break the clusters after they are detected as trimers. According to these results, a sintering temperature of 110 °C was chosen for our experiments.

3 Comparison of sub-population characterization with FACS and Image Analysis

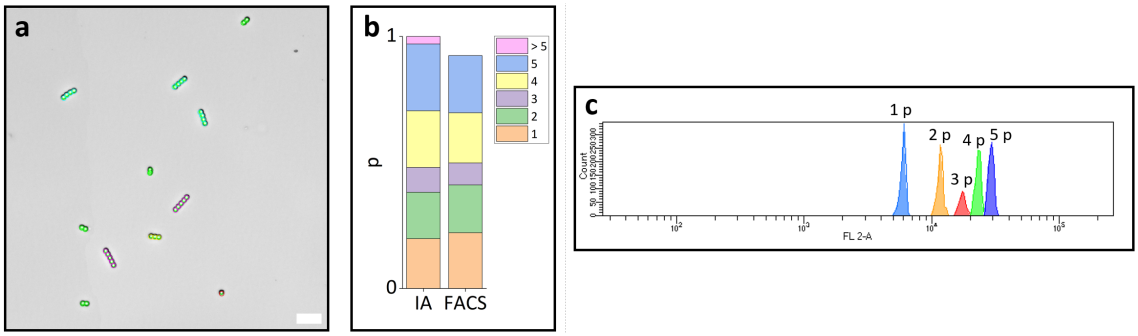

Figure S2: **Comparison of unsorted linear clusters (up to 5 particles) characterized by FACS versus image analysis** a) Image of the sample for the MatLab script analysis. Cluster of different particle numbers are indicated by color. b) Comparison of the sub-populations characterized by image analysis (IA) and FACS. c) Counts per fluorescence intensity of the unsorted sample obtained by FACS (same image as in Fig. 2). Scale bar is 20 µm.

4 Individual FACS: Red-Red, Red-green, and Green red dimers

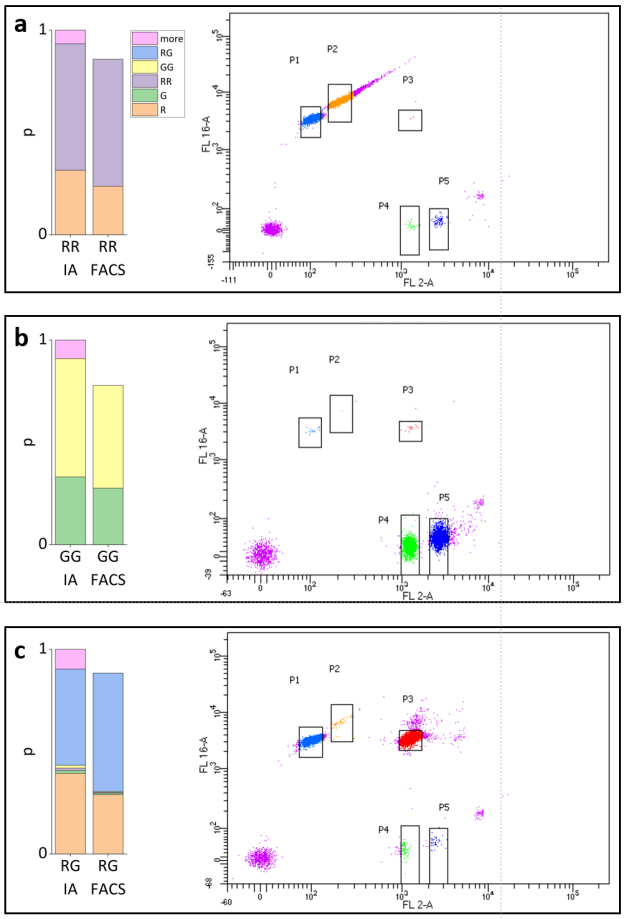

Figure S3: **Characterization of the red-red/green-green/red-green dimer samples before mixing.** a) Characterization of the red-red (RR) dimer sample. Left: Summary and comparison of image analysis and FACS. Right: Counts per fluorescence intensity of the red-red dimer sample obtained by FACS. b) Characterization of the green-green (GG) dimer sample. Left: Summary and comparison of image analysis and FACS. Right: Counts per fluorescence intensity of the green-green dimer sample obtained by FACS. c) Characterization of the red-green (RG) dimer sample. Left: Summary and comparison of image analysis and FACS. Right: Counts per fluorescence intensity of the red-green dimer sample obtained by FACS.

5 Individual FACS: 3-particle linear and triangle clusters

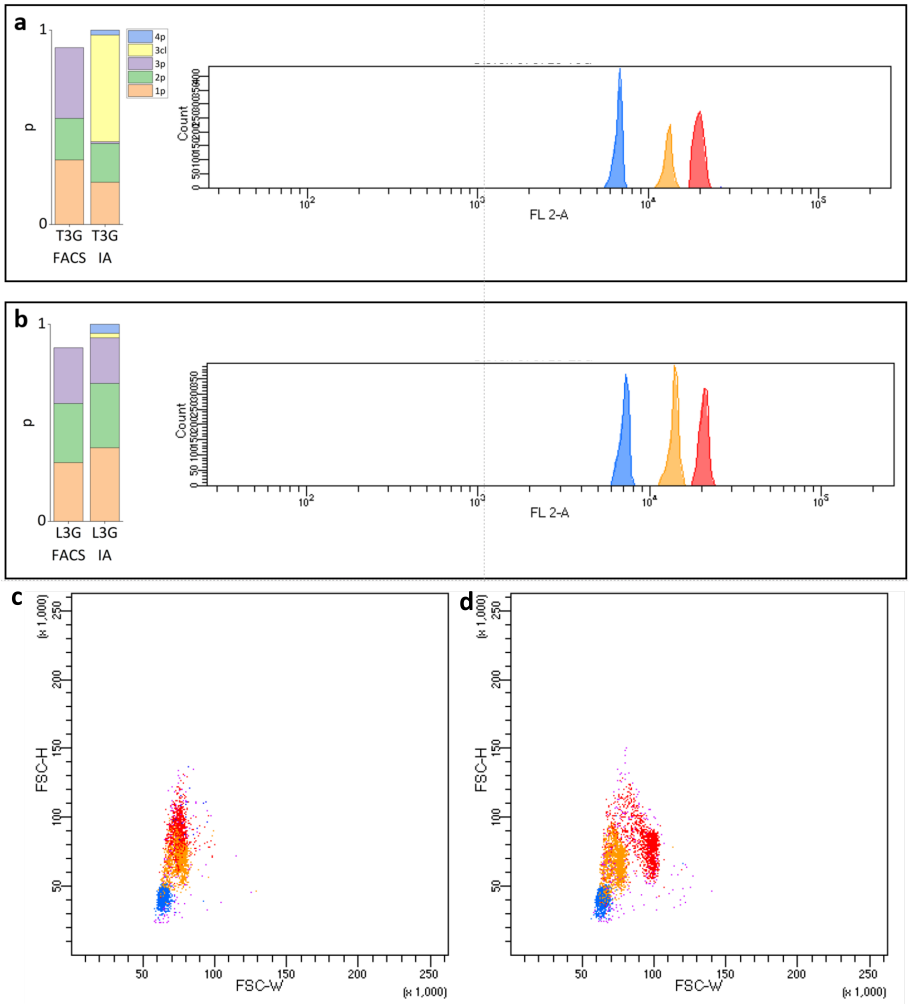

Figure S4: **Characterization of the individual linear and triangle 3-particle clusters samples before mixing.** a) Characterization of the triangle clusters. Left: Summary and comparison of image analysis and FACS. Right: Counts per fluorescence intensity of the triangle cluster sample obtained by FACS. b) Characterization of the linear clusters. Left: Summary and comparison of image analysis and FACS. Right: Counts per fluorescence intensity of the linear cluster sample obtained by FACS. c) Forward scattering height (FSC-H) versus Forward scattering width (FSC-W) for single particles (blue), dimers (orange), and trimers (red). d) Forward scattering height (FSC-H) versus Forward scattering width (FSC-W) of the linear cluster sample of single particles (blue), dimers (orange), and trimers (red).

6 Gates used for sorting

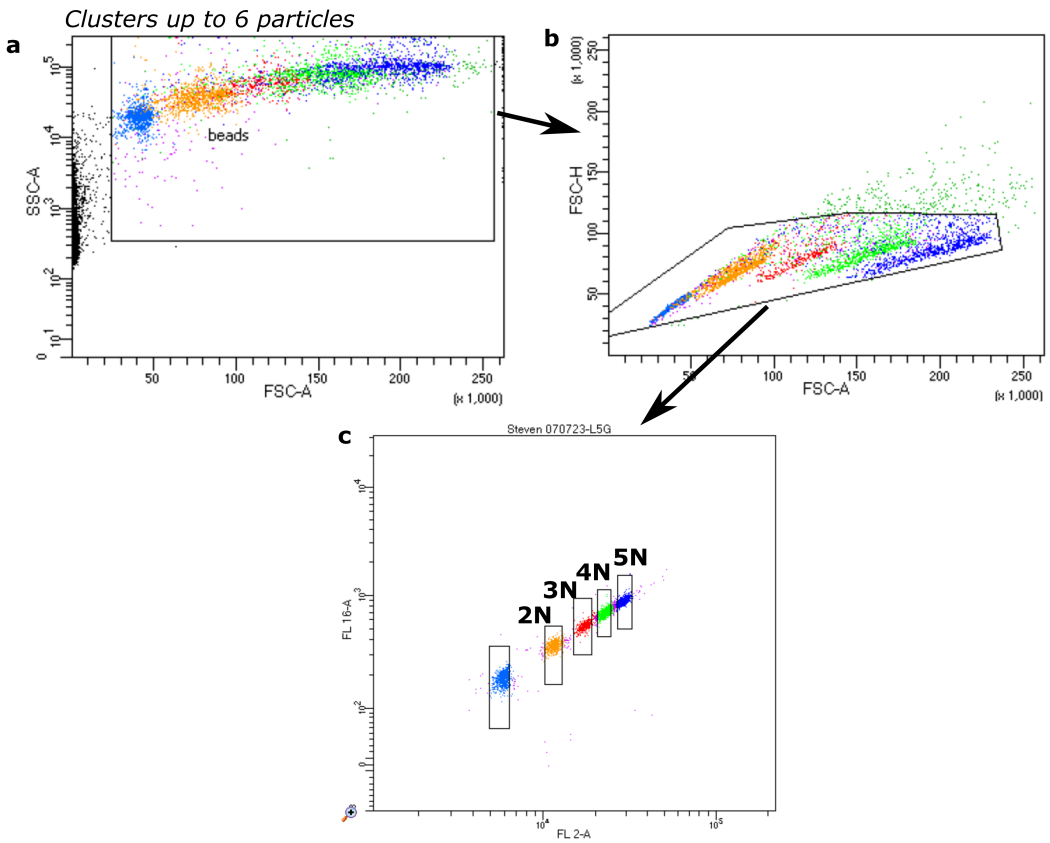

Figure S5: **Gates for sorting clusters up to 6 particles from sorting report a)** First gate: Side scattering (SSC-A) versus Forward scattering (FSC-A) for filtering debris. **b)** Second gate: Forward scattering height (FSC-H) versus Forward scattering area (FSC-A) to exclude very large aggregates. **c)** Third gate: Red (FL 16-A) versus Green fluorescence (FL 2-A) for sorting clusters from 2 to 5 particles (2-5N).

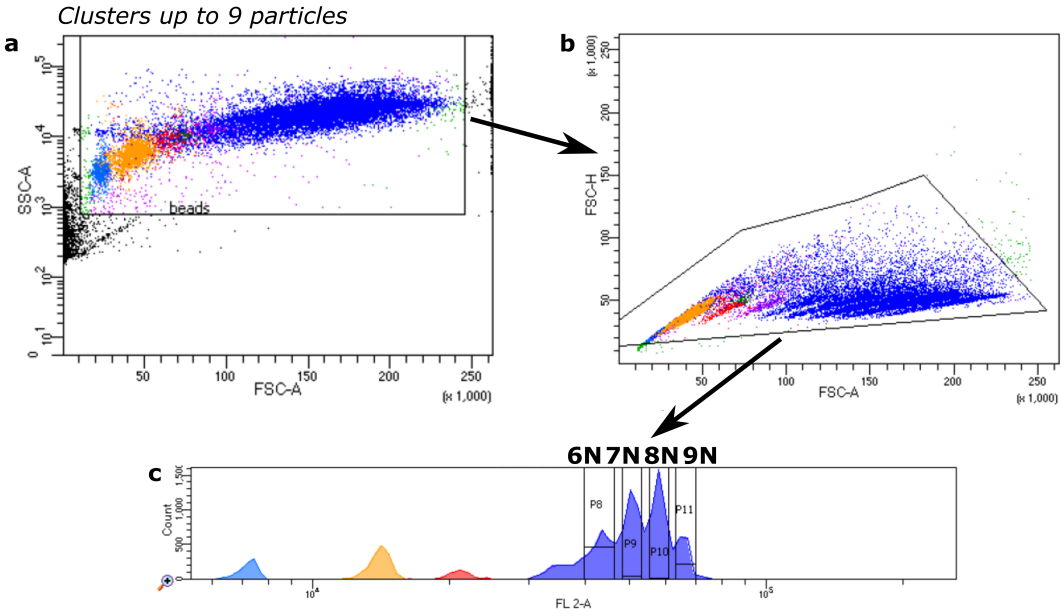

Figure S6: **Gates for sorting clusters with up to 10 particles from sorting report a)** First gate: Side scattering (SSC-A) versus Forward scattering (FSC-A) for filtering debris. **b)** Second gate: Forward scattering height (FSC-H) versus Forward scattering area (FSC-A) to exclude very large aggregates. **c)** Third gate: Green fluorescence (FL 2-A) for sorting clusters from 6 to 9 particles (6-9N).

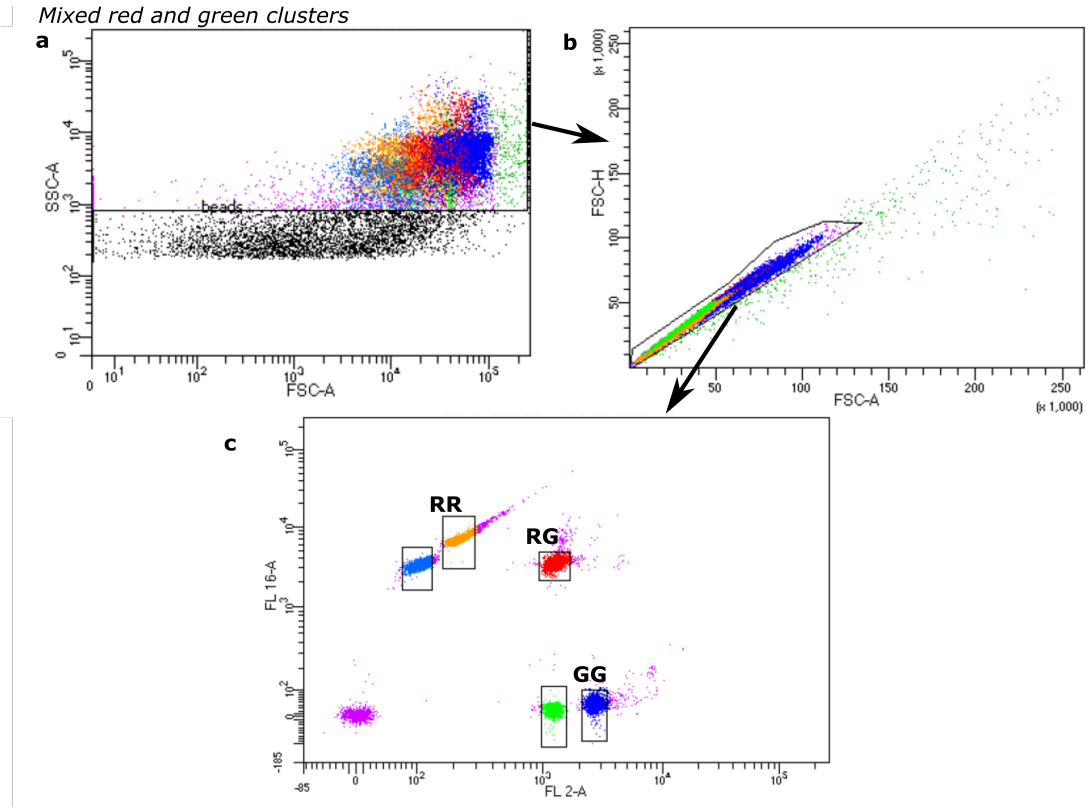

Figure S7: **Gates for sorting clusters of mixed red and green particles from sorting report** **a)** First gate: Side scattering (SSC-A) versus Forward scattering (FSC-A) for filtering debris. **b)** Second gate: Forward scattering height (FSC-H) versus Forward scattering area (FSC-A) to exclude very large aggregates. **c)** Third gate: Red (FL 16-A) versus Green fluorescence (FL 2-A) for sorting pure red fluorescent dimers (RR), pure green fluorescent dimers (GG), and dimers of one red and one green colloid (RG).

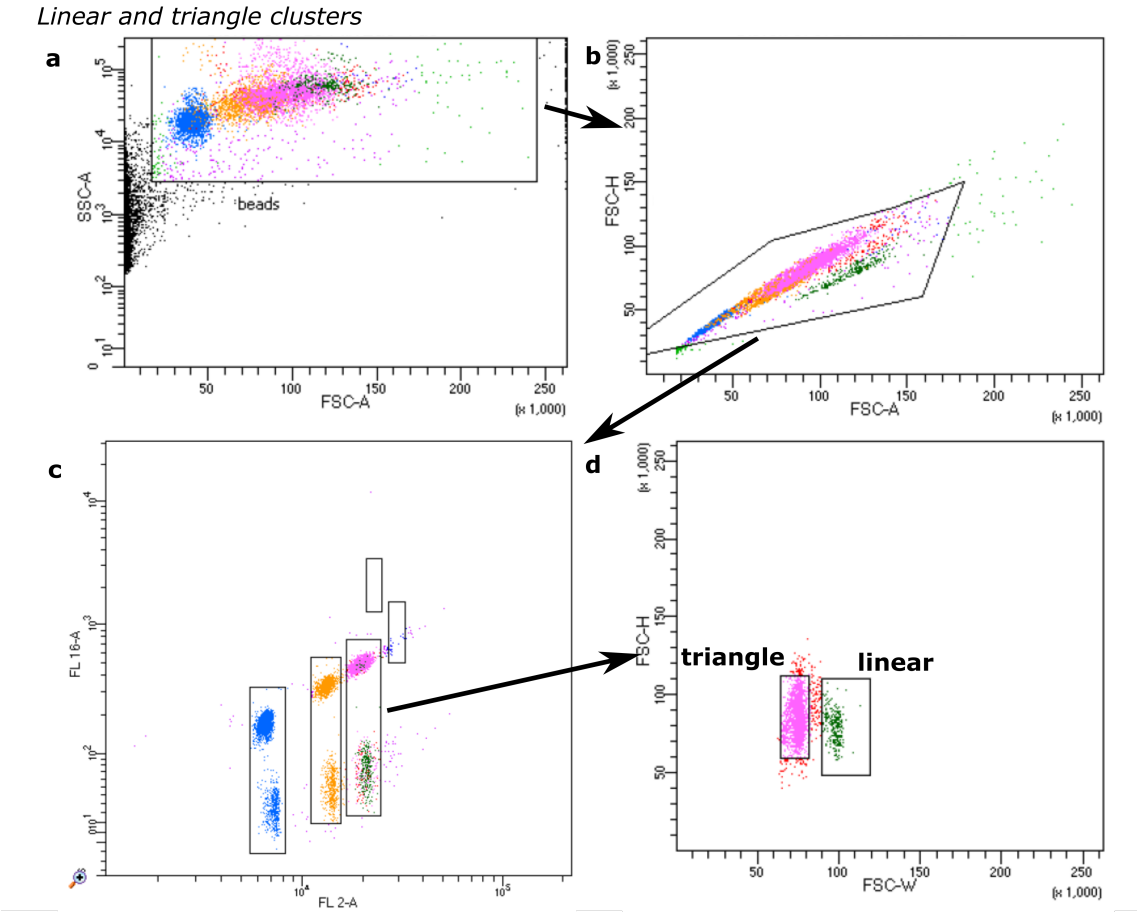

Figure S8: **Gates for sorting clusters based on shape a)** First gate: Side scattering (SSC-A) versus Forward scattering (FSC-A) for filtering debris. **b)** Second gate: Forward scattering height (FSC-H) versus Forward scattering area (FSC-A) to exclude very large aggregates.**c)** Third gate: Red (FL 16-A) versus Green fluorescence (FL 2-A) for sorting clusters 3 particle clusters. **d)** Fourth gate: Forward scattering height (FSC-H) versus Forward scattering width (FSC-W) to sort linear and triangular clusters.

References

[1] Songbo Ni, Jessica Leemann, Ivo Buttinoni, Lucio Isa, and Heiko Wolf. Programmable colloidal molecules from sequential capillarity-assisted particle assembly. *Science Advances*, 2(4):1–8, 2016.